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RESEARCH ARTICLE

The selection of intra-inter specific *Cucurbita* rootstocks for grafted melon seedlings

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ABSTRACT

This study was conducted to select promising Turkish local *Cucurbita* rootstocks among intraspecific and interspecific hybrids for grafted melon seedling based on hybrid success rate, hypocotyl traits and graft compatibility. Rooting potentials, root system architecture and plant growth parameters of the rootstocks grafted with melon were also evaluated. Two lines of *C. maxima* (male parent) and eleven lines of *C. moschata* (female parent) at S5 generation were used. In addition, six winter squash lines were used as male parent and six pumpkin lines were used as female parent in intraspecific hybridization program. Intraspecific hybrid combinations had quite high number of seeds per fruit as compared to interspecific hybrid combinations. The highest number of seeds per fruit was obtained from HMO2 × OMO2 (359) and OMO5 × HMO8 (314) hybrid combinations. Hypocotyl lengths of all hybrid cucurbit rootstock combinations ranged from 32.96 mm (RS17) to 73.35 mm (RS8) and hypocotyl thicknesses ranged from 2.71 mm (RS2) to 3.55 mm (RS8). Graft success rates varied between 96.6 – 100% in winter squash and pumpkin intraspecific hybrids and between 87.1 – 100% in interspecific hybrids. Significant differences were seen in root architecture parameters (root length, root volume, mean root diameter, root surface area and root dry matter weight) of intraspecific and interspecies hybrid *Cucurbita* lines. The findings of the study indicated that the intraspecific and interspecific local cucurbit rootstock candidates developed within the rootstock breeding program showed promise as viable options for commercial use as rootstocks for grafted melon seedlings. Research about the effects of selected intraspecific and interspecific hybrid *Cucurbita* rootstock candidates on earliness, fruit quality and yield parameters of grafted melon will continue.

Keywords: *Cucurbita maxima; Cucurbita moschata;* Rootstock breeding; Root system architecture; Melon

INTRODUCTION

Melon is a highly desired vegetable crop in the world. Global melon production was almost 28.5 million tons in 2020 and the main producing countries were China (13.9 million tons), Turkey (17.3 million tons) and Iran (12.8 million tons) (Faostat, 2020). Biotic and abiotic stress factors can reduce melon productivity worldwide. Indeed, vegetable grafting has gained significant attention in recent years for its ability to enhance plant resistance against both biotic and abiotic stressors, ultimately leading to improved yields in crop production. Melons are commonly grafted onto vigorous and disease-resistant rootstocks as a strategy for managing soil-borne pathogens (Bertucci et al., 2018). Certain rootstocks have been reported to enhance both growth and yield under various sub-optimal conditions. Indeed, studies by Ahn et al. (1999) and Zhou et al. (2007) have demonstrated the beneficial effects of certain rootstocks in improving growth and yield of plants under sub-optimal soil temperatures. Similarly, under reduced irrigation, Rouphael et al. (2008) demonstrated the favorable effects of specific rootstocks on growth, development and yield. Additionally, research by Colla et al. (2006) and Huang et al. (2010) has highlighted the ability of particular rootstocks to enhance growth and yield under salinity conditions.

Melon plants are commonly grafted onto various rootstocks, including interspecific hybrids of *C. maxima.* and *C. moschata*, intra *C. moschata* hybrids, and *Cucumis melo* rootstocks. It has also been reported to be grafted onto luffa. (King et al., 2010; Balkaya, 2014; Karaağaç, 2021). Interspecific hybrid squash rootstocks, particularly those derived from a cross between *C. maxima* and *C. moschata*, have been extensively used in melon grafting in order to enhance their growth and yield. These hybrid rootstocks possess several desirable traits that make them well-suited for melon production (Cohen et al., 2002; Edelstein et al.,

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2004; Bletsos, 2005; Karaağaç, 2013; Karaagac and Balkaya, 2013; Kobal Bekar et al., 2017).

Absolutely, the effects of grafting on plant performance are influenced by multiple factors, including the compatibility between rootstocks and scions, existing environmental conditions, and cultivation techniques (Koutsika-Sotirou and Traka-Mavrona, 2002; Fita et al., 2007). In the world, melon has a problem of incompatibility (Camalle et al., 2021; Xiong et al., 2021). In order to overcome this problem, new rootstocks without incompatibility problems should be developed by using new genetic resources. For this, intraspecies and interspecies hybridizations should be done. By grafting melon onto different Cucurbitaceae species melon, pumpkin, bitter gourd, bottle gourd, wax gourd, watermelon, cucumber, luffa), the researchers (Xiong et al., 2021) aimed to determine the compatibility of these rootstocks with melon scions. In the examination performed 42 days after grafting, it was determined that melons grafted on cucumber and zucchini compatible, while melons grafted onto bitter gourd, wax gourd, bottle gourd, luffa, and watermelon was incompatible. The study conducted by Edelstein et al. (2004) focused on investigating the compatibility between the scion, Melon (Cucumis melo L.) 'Arava,' and various experimental Cucurbita rootstocks. The researchers grafted the Melon scion onto 22 different rootstocks, which included both intra- and interspecific hybrids of the Cucurbita genus. According to the results of the study, the researchers reported that the compatibility, which is described as a strong development in greenhouse conditions, is an important parameter in rootstock preselection.

In terms of being economical, the first selection of proper rootstock criterion should be rootstock seed yield. Crosses between *C. maxima and C. moschata* often result in poor fruit set and low seed yield (Karaagac and Balkaya, 2013). For the success of grafted seedling production, another important factor is Hypocotyl properties of rootstocks. Different researchers have reported that the compatibility of candidate genotypes with different scions, and hypocotyl characteristics are important selection criteria in rootstock breeding programs (Cousins, 2005; King et al., 2010; Yıldız and Balkaya, 2016; Karaağaç et al., 2018). Oda et al. (1993) put forwarded that compatibility was not affected by the numbers of vascular bundles between cucumber scion and pumpkin rootstock, but smaller differences in the diameters of hypocotyls could potentially enhance compatibility.

In rootstock selection, the root structure should be strong along with the high seed yield and grafting success of the rootstock. Indeed, studies have demonstrated that a strong and vigorous root system of the rootstock plays a crucial role in the performance of grafted plants. The efficiency of water and nutrient uptake is increased by rootstocks that possess a vigorous root system architecture. As a result, this condition not only leads to increased yield but also aids in disease control (Lee, 1994; Balkaya, 2014; Karaağaç et al., 2018; Karaağaç, 2020). In recent years, researchers have directed their attention towards examining root system architectures as promising avenues for crop improvement (Saribaş et al., 2019; Karaağaç et al., 2020; Karaağaç, 2021). In rootstock breeding programs, one of the most challenging tasks is the specification of phenotypic variability in root characteristics. By examining RSA, researchers can gain insights into the spatial and structural arrangement of roots, which plays a crucial role in determining how plants interact with their environment (Ye et al., 2018). Therefore, the examination of root system architecture (RSA) through selected lines holds great potential and utility (Sheshshayee et al., 2011; Saribaş et al., 2019; Karaağaç, 2021).

In the world and in Turkey, the use of grafted seedlings in melon is lower than that of watermelon and cucumber. The reason for this can be shown as incompatibilities and thus the negative effect on yield and quality. China, Japan, and Korea have been at the forefront of initiating and leading the cucurbit rootstock breeding programs, but in Turkey, it is relatively new subject, with the current rootstock cultivars being older releases (Karaağac and Balkaya, 2013). In Turkey, rootstock breeding programmes are fewer in melons. By exploring and evaluating a wide range of melon rootstock candidates from germplasm collections, researchers can identify rootstocks with desirable traits (Özbahçe et al., 2021).

This study was conducted to choose promising rootstocks according to their crossing success ratio, hypocotyl traits and graft compatibility among intra *C. moschata* × *C. moshata* hybrids and interspecific *C. maxima* × *C. moschata* rootstock genotypes in rootstock breeding programs for grafted melon seedling. The study aimed to evaluate the relationship between hypocotyl morphology traits and graft compatibility, with the objective of identifying promising cultivars that possess favourable characteristics for use as melon rootstocks. It was also aimed to evaluate their rooting potentials and root architectures as rootstocks for melon. Furthermore, the study aimed to provide valuable insights into the future utilization of the selected rootstocks for the development of hybrid rootstocks in the production of grafted melon seedlings.

MATERIALS AND METHODS

This research was carried out in 3 stages in the provinces of Antalya and Samsun of Türkiye. In the first stage of the study, interspecific and intraspecific hybridizations were made between winter squash and pumpkin genotypes and suitable hybrid combinations were selected. In the

second stage, selected hybrid rootstock candidates were evaluated in terms of graft compatibility. In the last stage, performance of hybrid rootstocks in terms of plant biomass and root system architecture was evaluated. Entire *C. maxima* × *C. moschata* and C. *moschata* × *C. moschata* hybrid combinations were tested for *Fusarium oxysporum* f. sp. *melonis* race 1, 2, 3 by Prof. Dr. İsmail Erper and all combinations were found to be resistant to the disease.

Identification of suitable interspecific and intraspecific hybridization combinations

In this research, 2 inbred lines of *C. maxima* (Short vine and MA12) at S5 generation and 11 inbred lines of *C. moschata* (MO13Ş, HMO2, MO11YN, B1, OMO-5, AMO12, HMO8, OMO2, MO2, HMO-11, MO2H and B15) at S5 generation were used. In the interspecific crossing program, Short vine (SV) and MA12 winter squash genotypes were used as the female parent and 10 pumpkin genotypes were used as the male parent. In the intraspecific crossing program, 6 pumpkin genotypes were used as the male parent and 6 pumpkin genotypes were used as the female parent (Table 1).

In the initial stage, the seeds of various pumpkin species genotypes were planted on May 15, 2018. Peat and perlite ratio of 2:1 were used as seed sowing medium. Twenty seedlings from each genotype were transplanted at the 4-5 leaves stage in the breeding greenhouse of Genetika Seed Company in Antalya. The spacing between the plants was set at 2.8 meters × 2.0 meters. The plants were consistently protected and managed throughout the entire growing season to ensure optimal growth and development.

All interspecific and intraspecific hybridization stages (Fig. 1). were made according to the Karaağaç (2021). Cultural practices (irrigation, fertilization, etc.) in the greenhouse were continued until the fruits developed (Karağaç, 2013). Developed fruits were harvested 65-75 days after hybridization. Seed harvests were made from the fruits that were physiologically matured. Number of seeds per fruit, number of abortive seeds per fruit and normal number of seeds per fruit were determined. Successful combinations (eight interspecific and seven intraspecific hybrids) were used as material in the next experiment.

Determination the survival rates of the interspecific and intraspecific rootstock genotypes

Experiments were continued with the selected hybrid genotypes based on number of seeds. Experiments were conducted with 90 seeds in randomized blocks design with three replications (30 seeds per replication) of each rootstock genotypesThe scions were planted in plastic trays filled with a mixture of peat and perlite in a ratio of 2:1 (v/v). Seven *C. moschata* and eight *C. maxima* × *C. moschata* rootstock genotypes and control rootstock cultivars (Gürdal, Acar and TZ-148 *C. maxima* × *C. moschata* rootstocks) were grafted with the Pandora F1 melon cultivar using the splice grafting method on 25th of March 2019 (Davis et al., 2008). All grafted plants were placed in a growth chamber covered with a plastic film. The grafted plants were exposed to the environment with 95% relative humidity and temperatures ranging from 25 to 30 °C. The grafting survival rate was assigned by calculating the percentage of healthy seedlings 15 days after grafting (Karaağaç, et al. 2018). The seedlings which have proper shoot androot growth and no incompatibility were evaluated as healthy.

$$\text{Survival rate} = \frac{\text{Number healty seedlings}}{\text{Total number of grafted seedlings}} * 100$$

Determination the plant growth and root system architecture of rootstock combinations

Experiments were carried out in randomized blocks design three replications (each replication comprised of 5 plants) in a temperature-controlled polycarbonate greenhouse in Samsun province. Both grafted and non-grafted plants (Pandora F1) were planted in 7-liter pots (a mixture of peat

Table 1: Interspecific (*C. maxima*×C. *moschata*) and intraspecific (*C. moschata* × *C. moschata*) hybrid combinations.

Interspecific hybrid combinations (*C. maxima* x C. *moschata*)			Interspecific hybrid combinations (*C. maxima* x C. *moschata*)			Intraspecific hybrid combinations (*C. moschata* x *C. moschata*)		
Female		Male	Female		Male	Female		Male
Short vine (SV)	×	HMO2	MA12	×	HMO2	B1	×	MO13Ş
	×	HMO-11		×	HMO-11	B15	×	MO13Ş
	×	HMO-8		×	HMO-8	B1	×	MO11YN
	×	AMO-12		×	AMO-12	MO2	×	MO2H
	×	OMO-5		×	OMO-5	OMO2	×	HMO2
	×	B1		×	B1	OMO5	×	HMO2
	×	MO13Ş		×	MO13Ş	OMO2	×	HMO11
	×	B15		×	B15	HMO8	×	OMO5
	×	MO11YN		×	MO11YN			
	×	MO2		×	MO2			

and perlite in a ratio of 2:1 was used as medium) on 6th of May 2019 and grown for 30 days (Sallaku et al., 2022). Then, the plants were removed from pots and leaves, roots and stems were separated. The roots washed and dry weights of the plant parts were determined by drying at 70 °C for 72 hours. In this study, root development was examined in three periods, 10, 20 and 30 days after planting seedlings into the pots. The following method was used to examine the root system architecture of rootstock genotypes. Roots were scanned using an Epson Expression 10000XL scanner at a resolution of 400 dpi. The WinRHIZO software was employed to analyze the scanned images and determine root diameter (mm), total root length (m), root volume, and root surface area (cm^2).

Statistical analysis

The experimental data underwent analysis of variance using SAS-JMP statistical software. To stated significant differences between means, Tukey's multiple range test was utilized to determine the differences between the means of different groups.

RESULTS AND DISCUSSION

Suitable interspecific and intraspecific hybridization combinations

Interspecies hybrid combinations between winter squash and pumpkin genotypes were evaluated in detail for the number of seeds per fruit. Successful hybrid fruit was not obtained from only one hybrid combination (MA12 × HMO2). Number of seeds per fruit varied between 22 - 138 in combinations in which Short vine genotype was used as female parent and between 50 - 157 in combinations in which MA12 was used (Table 2). The highest number of seeds per fruit was obtained from hybrid combinations MA12 × MO2 (157) and Short vine × HMO2 (138). In interspecies hybridizations, combinations in which MA12 was used as female parent yielded greater number of seeds than the combinations in which short vine was used as parent. The highest number of abortive seeds was found in the hybrid combinations of Short vine x OMO5 (86 seeds) and Short vine x HMO11 (77 seeds), followed by MA12 × OMO5 (60) combination. Present finding revealed that number of abortive seeds was generally lower in hybrid fruits of MA12 × *C. moschata* combinations (Table 2). It was determined that number of seeds per fruit was not sufficient alone as a criterion in chestnut squash and pumpkin interspecies hybrid combination selection and greater attention should be paid to number of normal seeds. In terms of normal seed yield, MA12 × MO13Ş (116 seeds/fruit) and Short vine × HMO2 (102 seed/fruit) showed the highest performance. Normal seeds were not obtained from Short vine × HMO8, Short vine × OMO5, Short vine × B15 and Short vine × MO11YN hybrids (Table 2). In interspecies hybridizations carried out in breeding programs, generally low outcomes for hybrid success

Fig 1. Hybridization stages of *C. maxima* × *C. moschata* genotypes.

Table 2: Number of seeds from intraspecific and interspecific hybrid combinations.

Interspecific hybrid combinations (*C. maxima* × *C. moschata*)						
Female		**Male**	**Number of seeds/fruit**	**Number of abortive seeds/fruit**	**Number of normal seeds/fruit**	**Normal seed rate/fruit**
Short vine	×	HMO2	138	36	102	73.91
	×	HMO11	89	77	12	13.48
	×	HMO8	35	35	0	0
	×	AMO12	90	44	46	51.11
	×	OMO5	86	86	0	0
	×	B1	57	52	5	8.77
	×	MO13Ş	38	28	10	26.32
	×	B15	27	27	0	0
	×	MO11YN	22	22	0	0
	×	MO2	57	45	12	21.05
Mean			63.9±36.52	45.2±21.28	18.7±32.38	18.70±26.52
MA12	×	HMO2	Could not obtain hybrid fruit			
	×	HMO11	108	15	93	86.11
	×	HMO8	63	31	32	50.79
	×	AMO12	50	35	15	30.00
	×	OMO5	105	60	45	42.86
	×	B1	66	38	28	42.42
	×	MO13Ş	137	21	116	84.67
	×	B15	52	18	34	65.38
	×	MO11YN	61	15	46	75.41
	×	MO2	157	47	110	70.06
Mean			88.78±39.41	31.11±15.58	57.67±30.09	60.86±20.12
Intraspecific hybrid combinations (*C. moschata* × *C. moschata*)						
Female		**Male**	**Number of seeds/fruit**	**Number of abortive seeds/fruit**	**Number of normal seeds/fruit**	**Normal seed rate/fruit**
B1	×	MO13Ş	154	6	148	96.10
B15	×	MO13Ş	187	24	163	87.17
B1	×	MO11YN	86	22	64	74.42
MO2	×	MO2H	252	8	244	96.83
OMO2	×	HMO2	359	32	327	91.09
OMO5	×	HMO2	220	15	205	93.18
OMO2	×	HMO11	72	30	42	58.33
HMO8	×	OMO5	314	18	296	94.27
Mean			205.5	19.38	186.13	86.42

rates are obtained depending on the parents (mostly as a result of either the pollen tube or the cessation of embryo development) (Karaağaç, 2013). Loy (2012) reported low success rates for interspecies hybridizations between *C. maxima* × *C. moschata* and indicated that fruit and seeds were not usually obtained. However, it was stated in many studies that sufficient seeds could be obtained (Hayase, 1956; Uretsky, 2012; Karaağaç and Balkaya, 2013). Bemis and Nelson (1963) could not obtain positive outcomes from 20 out of 43 interspecies hybrid combinations. The highest success (335 seeds) was obtained from *C. maxima* × *C. moschata* combination. Yongan et al. (2002a) worked on 12 different combinations of interspecies hybrids of *C. maxima* × *C. moschata* and was able to obtain seeds only from 3 combinations. In that study, success of interspecies hybrids varied considerably depending on the parents of combinations. Various treatments are practiced to increase the success rate in interspecies hybridizations and to obtain strong and healthy hybrid plants. These practices include selection of genotypes with high hybrid compatibility (Whitaker and Robinson, 1986; Karaağaç and Balkaya, 2013), bridge crossings, bud pollination (Hayase, 1961; Yongan et al., 2002a), repetitive pollination (Yongan et al. 2002b) and use of growth regulators (Nascimento et al., 2007).

In pumpkin rootstocks, number of seeds in the hybrid fruit should be quite high (Karaağaç, 2013). In this study, it was determined that number of seeds per fruit was quite high in intraspecific hybrid combinations as compared to interspecific hybrid combinations (Table 2). The highest number of seeds per fruit was obtained from hybrid combinations of HMO2 × OMO2 (359) and OMO5 × HMO8 (314). The highest number of normal seeds was

also obtained from the same combinations (327 and 296 seeds, respectively). It was determined that number of abortive seeds in intraspecific hybrids was low and varied between 6-32, largely depending on parents. The lowest number of abortive seeds was determined in MO13Ş x B1 combination (6 seeds). In terms of normal seed yield, combinations of MO2H × MO2 (96.8%) and MO13Ş × B1 (96.1%) showed the highest performance.

A total of 28 hybrid combinations (14 of *C. maxima* × *C. moschata* and 14 of *C. moschata* × *C. moschata*) were made in this study and 15 combinations (8 from *C. maxima*, *C. moschata* and 7 from *C. moschata* × *C. moschata*) were selected as rootstock candidates based on the ratio of normal seeds in hybrid fruit.

The survival rates of the interspecific and intraspecific rootstock genotypes

Before grafting, hypocotyl length and thickness of pumpkin genotypes were measured. It was determined that genotypes were significantly different in terms of hypocotyl characteristics (Table 3).

The hypocotyl lengths of all hybrid rootstock combinations ranged from 32.96 mm (RS17) to 73.35 mm (RS8). In winter squash and pumpkin interspecies hybrid rootstocks, hypocotyl lengths were determined as 51.26 (RS9) and 73.35 mm (RS) in short vine genotype (female parent) hybrids. In MA12 (female parent) hybrids, hypocotyl lengths varied between 32.96 (RS17) - 59.02 mm (RS12). It was determined that pumpkin rootstocks generally had longer hypocotyl lengths (45.69 - 64.93 mm) than interspecies hybrids. Hypocotyl lengths of commercial pumpkin rootstocks (RS18, RS19 and RS20) used as control in the experiment were measured as between 41.92 - 55.41 mm. The hypocotyl length of Pandora F1 melon variety used as scion was measured as 40.07 mm. Of the local cucurbit rootstocks, only RS17 (32.96 mm), RS15 (33.13 mm) and RS16 (33.57 mm) produced hypocotyl shorter than the scion. Karaağaç (2013) worked on squash genotypes and determined the shortest hypocotyl length (27.31 mm) in K9 genotype and the longest hypocotyl length (38.38 mm) in K3 genotype. Yetişir and Sari (2004) determined that hypocotyl lengths of different cucurbit species varied between 2.0 cm (*Luffa cylindrica* and *Benincasa hispida*) and 6.9 cm (*Lagenaria* spp.). It was determined that pumpkin genotype had a hypocotyl length of 2.9 cm and winter squash genotype had a hypocotyl length of 3.3 cm. Yetişir et al. (2007) measured hypocotyl lengths of squash rootstocks as between 2.35 and 5.70 cm.

Similarly, in present study hypocotyl lengths of cucurbit rootstock combinations were found to be between 32.96 - 73.35 mm. In grafting experiments, it has been observed that hypocotyl thickness plays a more significant role in determining grafting success rates compared to hypocotyl length. Indeed, while hypocotyl thickness is important for grafting success, it is also desirable to have a hypocotyl that is not too short. there are some potential disadvantages associated with long hypocotyls during

Table 3: Hypocotyl characteristics and graft success rates of different rootstocks (CR: Commercial rootstock)

Rootstock Code	Rootstock		Hypocotyl length (mm)	Hypocotyl thickness (mm)	Rootstock-scion thickness difference	Survival rates (%)
RS1	Intraspecific rootstock	B1 × MO13Ş	46.37^{g}	3.23^{a-d}	0.05	100.0
RS2		B15 × MO13Ş	56.20^{d}	2.71^{e}	-0.47	96.6
RS3		B1 × MO11YN	45.69^{g}	3.33^{ab}	0.15	100.0
RS4		MO2 × MO2H	49.14^{f}	2.93^{de}	-0.25	97.4
RS5		OMO2 × HMO2	52.14^{e}	3.21^{b-d}	0.03	100.0
RS6		OMO5 × HMO2	48.34^{f}	2.93^{de}	-0.25	100.0
RS7		HMO8 × OMO5	64.93^{b}	3.42^{ab}	0.24	100.0
RS8	Interspecific rootstock	Short vine × HMO2	73.35^{a}	3.55^{a}	0.37	100.0
RS9		Short vine × AMO12	51.26^{e}	3.50^{ab}	0.32	100.0
RS11		MA12 × HMO11	45.95^{g}	3.45^{ab}	0.27	100.0
RS12		MA12 × HMO8	59.02^{c}	3.32^{ab}	0.14	100.0
RS14		MA12 × MO13Ş	40.69^{hi}	3.44^{ab}	0.26	87.1
RS15		MA12 × B15	33.13^{j}	2.97^{c-e}	-0.21	88.6
RS16		MA12 × MO11YN	33.57^{j}	2.95^{de}	-0.23	100.0
RS17		MA12 × MO2	32.96^{j}	3.42^{ab}	0.24	100.0
RS18	CR	Gürdal	55.41^{d}	3.24^{a-d}	0.06	89.2
RS19		Acar	52.72^{e}	2.95^{de}	-0.23	100.0
RS20		TZ-148	41.92^{h}	3.27^{a-c}	0.09	100.0
Scion		Pandora	40.07^{i}	3.18^{b-d}		100.0
P			≤0.01	≤0.01		
%CV			2.18	5.20		

Table 4: Biomass characteristics of melon plants grafted with interspecific and intraspecific pumpkin rootstocks 30 days after planting (CR: Commercial Rootstock)

Rootstock Code		Rootstock	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)
RS1	Intraspecific Rootstock	B1 × MO13Ş	3.10^{ab}	1.54^{ab}	0.65^{ab}
RS2		B15 × MO13Ş	2.98^{a-c}	1.21^{a-c}	0.60^{bc}
RS3		B1 × MO11YN	2.49^{a-d}	1.18^{a-c}	0.35^{g}
RS4		MO2 × MO2H	2.59^{a-d}	1.23^{a-c}	0.58^{b-e}
RS5		OMO2 × HMO2	2.81^{a-d}	1.46^{a-c}	0.57^{b-f}
RS6		OMO5 × HMO2	2.17^{b-d}	0.96^{cd}	0.34^{g}
RS7		HMO8 × OMO5	2.09^{cd}	1.27^{a-c}	0.50^{b-g}
RS8	Interspecific Rootstock	Short vine × HMO2	2.61^{a-d}	1.22^{a-c}	0.41^{d-g}
RS9		Short vine × AMO12	2.16^{b-d}	1.07^{b-d}	0.47^{c-g}
RS11		MA12 × HMO11	3.05^{a-c}	1.38^{a-c}	0.46^{c-g}
RS12		MA12 × HMO8	3.03^{a-c}	1.55^{ab}	0.77^{a}
RS14		MA12 × MO13Ş	2.46^{a-d}	1.31^{a-c}	0.53^{b-f}
RS15		MA12 × B15	2.08 ^{d}	1.13^{a-c}	0.40^{e-g}
RS16		MA12 × MO11YN	3.09^{ab}	1.62 a	0.59^{b-d}
RS17		MA12 × MO2	2.49^{a-d}	1.07^{b-d}	0.49^{b-g}
RS18	CR	Gürdal	2.13^{b-d}	1.12^{a-d}	0.45^{c-g}
RS19		Acar	3.24^{a}	1.31^{a-c}	0.59^{bc}
RS20		TZ-148	2.41^{a-d}	1.24^{a-c}	0.57^{b-f}
Scion		Pandora	1.20^{e}	0.61^{d}	0.39^{fg}
P			≤0.01	≤0.05	≤0.01
%CV			19.35	20.54	17.97

grafting. When the hypocotyl is longer, it may be leading to instability and potential failure of the graft union. Additionally, the grafting apparatus or clips used to hold the graft may need to be attached from a higher point on the hypocotyl, which can cause the grafted plant to lean or lie on its side, affecting its overall stability. Hypocotyl lengths can be controlled with regulating environmental factors, and applying different chemicals (Hamamoto and Oda, 1997; Yang et al., 2012).

In all hybrid cucurbit rootstock combinations, hypocotyl thicknesses varied between 2.71 mm (RS2) and 3.55 mm (RS8) (Table 3). In winter squash and pumpkin interspecies hybrid rootstocks, hypocotyl thicknesses varied between 3.50 mm (RS9) and 3.55 mm (RS8) in Short vine genotype (female parent) hybrids and between 2.95 (RS16) and 3.45 mm (RS11) in MA12 (female parent) hybrids. Hypocotyl thicknesses of commercial cucurbit rootstocks (RS18, RS19 and RS20) used as control ranged between 2.95 and 3.27 mm and hypocotyl thickness of Pandora F1 melon variety used as scion was measured as 3.18 mm. Edelstein et al. (2004) examined hypocotyl and vascular bundles in Arava F1 melon cultivar grafted on 22 different cucurbit rootstocks and reported that hypocotyl thickness of the rootstocks varied between 3.6 - 6.7 mm and there was no relationship between the number of vascular bundles and hypocotyl thickness of rootstocks and plant vigor. Karaağaç (2013) reported hypocotyl thickness of winter squash, pumpkin and winter squash x pumpkin hybrid rootstocks as between 3.10 and 4.97 mm. Present findings comply with those earlier ones.

Karaağaç (2013) stated that there was a positive correlation between the small difference between rootstock and scion hypocotyl thicknesses and the high success rate of grafting. In this study, the differences between hypocotyl thicknesses of rootstock and scion in the grafting region were determined. The lowest thickness difference was found in RS5 (0.03 mm), RS1 (0.05 mm), Gürdal (0.06) and TZ-48 (0.09) rootstocks. The greatest thickness difference was seen in RS2 (-0.47 mm) mm, RS8 (0.37 mm) and RS9 (0.32 mm) rootstocks (Table 3). Salehi et al. (2009) investigated the rootstock and scion hypocotyl thickness differences in Khatooni melon cultivar grafted on *C. maxima* × *C. moschata* (Shinto Hongto, Shintozwa, Ace) rootstocks and reported the lowest hypocotyl thickness difference (0.14 mm) for Khatooni/Shinto Hongto combination.

Grafting success rates of intraspecific pumpkin hybrids ranged from 96.6 to 100%. A 100% graft success rate was achieved in RS1, RS3, RS5, RS6 and RS7 pumpkin hybrid rootstocks. The lowest grafting success in this group was determined in RS2 rootstock. Grafting success rates of winter squash and pumpkin interspecies hybrids varied between 87.1 - 100% (Table 3). In commercial rootstocks, success rates varied between 89.2% (Gürdal) - 100% (Acar and TZ 148). Quite different and variable success rates have been reported in many studies on grafting success rates of cucurbits. Salehi et al. (2009) stated that the average graft

success of rootstock/scion combinations in Khatooni melon cultivar grafted on *C. maxima* × *C. moschata* (Shinto Hongto, Shintozwa, Ace) rootstocks was 99.7% and there was no significant difference between the rootstocks. Traka-Mavrona et al. (2000) grafted the local cultivar "Kalkabaki" of the genus *C. moschata* and rootstocks of *C. maxima* × *C. moschata* (TZ-148, Mamouth) with different melon cultivars (Thraki, K. Banana, L. Amynteou, Peplo). The graft compatibility rates of *C. maxima* × *C. moschata* rootstocks varied between 42 - 91%. In the Kalkabaki/ Thraki combination, on the other hand, graft compatibility did not occur. In another study carried out to develop melon rootstocks by using the local genotypes of Greece, *C. moschata* cv. Kalkabaki and *C. maxima* cv. Kolokitha local varieties were used as rootstocks and *C. melo* cv. Lefko Amynteou and Thrakiotiko local melon varieties were used as scions. Kalkabaki variety, one of the local cucurbit rootstocks, showed low graft compatibility (7 - 34%). In Kolokitha winter squash, graft success rate varied between 79 - 96% (Koutsika-Sotiriou et al., 2004). Grafting success in vegetables vary with the adaptability of the rootstock, grafting method applied, grafting ability and post-grafting care conditions (Hassell et al., 2008; Jang et al., 2011; Yang et al., 2012; Balkaya, 2014). Since different genetic materials and grafting methods were used in this and the other studies, success rates of the examined rootstocks differed significantly.

Biomass and root system architecture of rootstock combinations

The root system, which connects plants to the environment in which they grow, plays an important role in plant growth and productivity, especially under stress conditions, by significantly affecting some physiological events such as water and nutrient intake. Therefore, in recent years, investigation of root architectures and phenotypic selection of rootstocks in many vegetable species have been included in breeding programs (Bertucci et al., 2018; Sarıbaş et al., 2019; Karaağaç, 2020; Kanal et al., 2021). In the experiment, root system architecture parameters of 19 genotypes (15 rootstock candidates, 3 commercial rootstocks and scion) included in the melon rootstock breeding program, there were significant differences.

The total root lengths of cucurbit rootstocks determined on the 10th, 20th and 30th days after planting varied between 124.48 - 192.00 cm, 840.45 - 1394.44 cm and 1199.87 - 2041.94 cm, respectively (Fig. 2). It is observed that the rate of root length increase of the rootstocks was quite slow in the first 10 days after planting, a high rate of increase was recorded in the period between the 10th and 20th days and the rate of root length growth slowed down again in the period between the 20th and 30th days (Fig. 2). During the first 10-day period from the planting, average root length was measured as 158.53 cm in rootstock candidates (RS1-RS17) and 160.93 cm in commercial rootstocks (RS18-RS20). However, the average root lengths the rootstock candidates on 20th day (1268.58 cm) and 30th day (1658.82 cm) were higher than those of the commercial rootstocks (1114.56 cm and 1258.28 cm). The average root length of rootstock candidates on 30th day was 31.83% longer than the commercial rootstocks. On the 30th day from the planting of intraspecific hybrid rootstocks (RS1-RS7), the highest root length was determined in RS2 (2041.94 cm) and RS1 (1951.88 cm) genotypes and the lowest root length was determined in RS4 (1398.29 cm) genotype. In winter squash and pumpkin interspecies hybrid rootstock genotypes, the highest root length was seen in RS9 (1972.48 cm) genotype and the lowest root length was recorded in TZ-148 commercial rootstock (RS20; 1199.87 cm). TZ-148 was followed by RS17 rootstock candidate (1260.31 cm) and commercial rootstocks Acar (RS19; 1274.96 cm) and Gürdal (RS18; 1297.98 cm). The total root length of Pandora F1 melon variety used as a scion was 1272.23 cm. It was determined that the average root lengths of intraspecific pumpkin rootstocks determined on the 30th day were higher than winter squash and pumpkin hybrid rootstocks and commercial rootstocks. In plant production, especially under biotic and abiotic stress conditions, it is desired that the total root length was also higher in order for the roots to go deep and have a good water and nutrient uptake (Krasilnikoff et al., 2003; Lambers et al., 2006; Comas et al., 2013; Özgen and Balkaya, 2022).

The root diameter is closely related to root hair density and length. It was noted that low average root diameter affected the absorption ability of the root positively (Eissenstat, 1992). Lovelli et al. (2012) reported that mean root diameter decreased and root length ratio increased, which in turn increased the water and nutrient utilization efficiency, especially in stressful conditions. Root diameter of the rootstocks varied between 0.83 mm (RS18) and 1.62 mm (RS4) on the 10th day after planting, between 1.38 mm (RS8) and 2.48 mm (RS20) on the 20th day and between 1.61 mm (RS8) and 2.62 mm (RS12) on 30th day (Fig. 3).

The root diameters measured on the 30th day after planting were significantly different ($P \leq 0.01$). The highest average root diameters were measured in RS12 (2.62 mm), RS20 (2.50 mm), RS16 (2.45 mm) and RS4 (2.43 mm) rootstocks and the lowest average root diameters in RS8 (1.61 mm), RS9 (1.70 mm) and RS1 (1.78 mm) rootstocks. The average root diameter of Pandora F1 melon variety used as a scion (1.5 mm) was lower than that of cucurbit rootstocks. The average root diameter was 2.03 mm in intraspecific hybrid rootstocks, 2.14 mm in

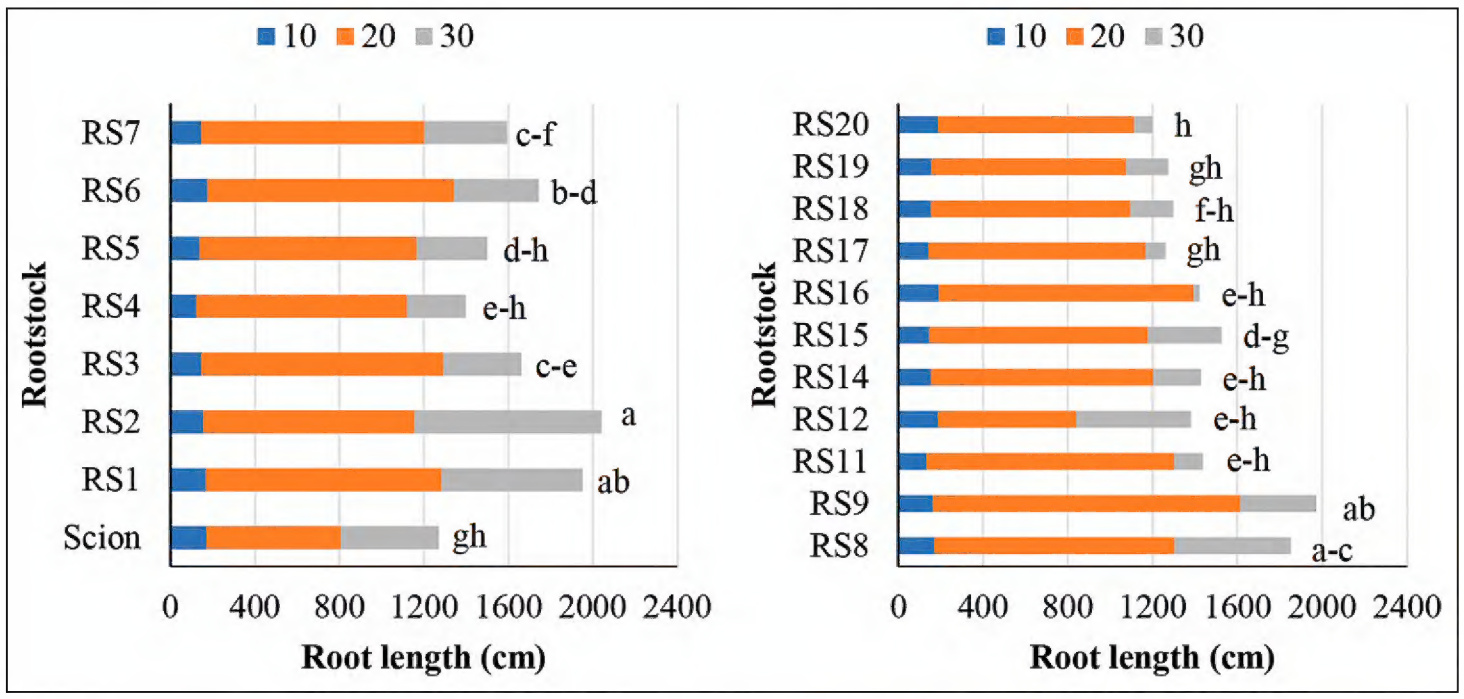

Fig 2. Root length (cm) of intraspecific (left) and interspecific (right) pumpkin rootstocks 10, 20 and 30 days after planting.

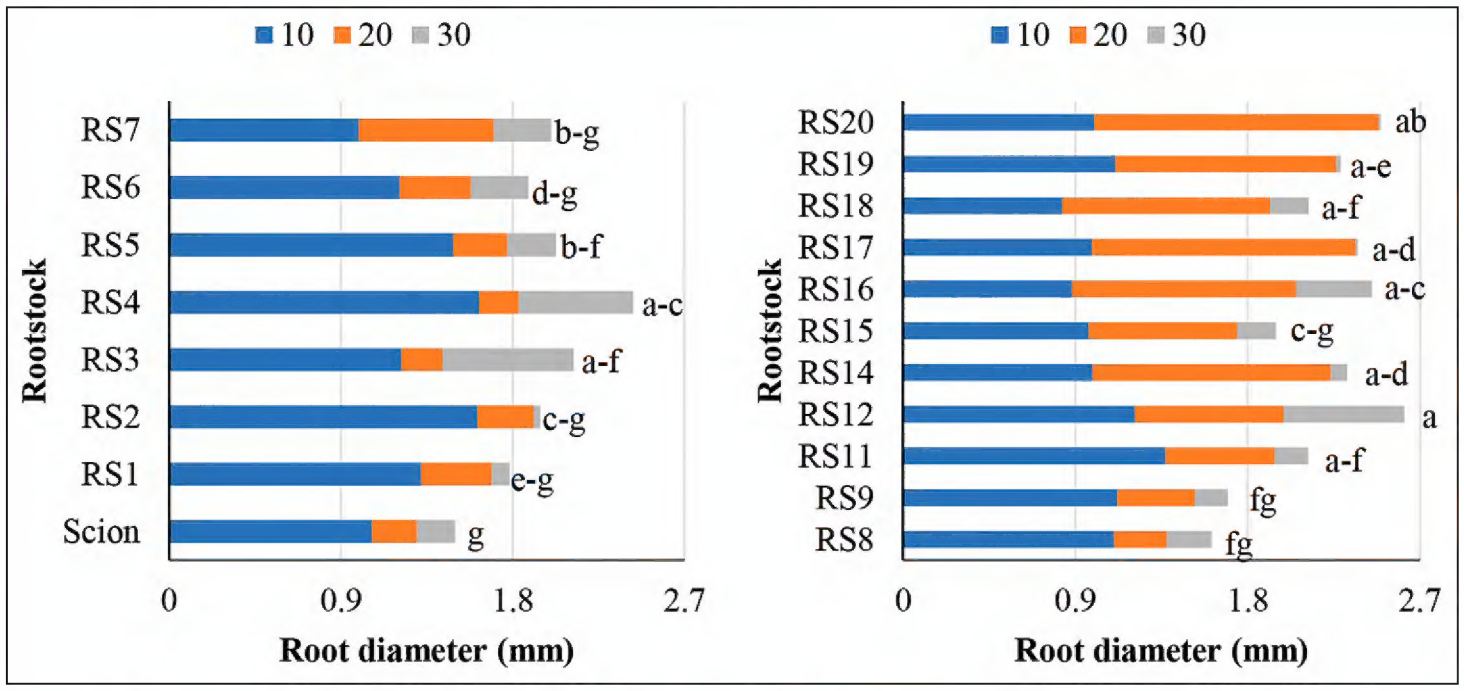

Fig 3. Root diameter (mm) of intraspecific (left) and interspecific (right) pumpkin rootstocks 10, 20 and 30 days after planting.

interspecies hybrid rootstocks and 2.30 mm in commercial rootstocks. Rootstock candidates showed lower mean root diameter than commercial rootstocks. In intraspecific hybrid rootstocks and scion, root diameters increased more in the first 10-day period and the rate of increase decreased in the next 10-day period. In interspecies hybrid rootstocks, mean root diameters increased rapidly in 10-day periods up to the 20th day and decreased in the last 10-day period (Fig. 3).

A larger root surface area provides more opportunities for the root system to explore and extract resources from the soil. It increases the overall absorptive capacity of the roots, enabling efficient uptake of water and essential nutrients. Lovelli et al. (2012) reported that water and nutrient uptake became easier and yields increased accordingly with the increase of root surface area. There were significant ($P \leq 0.01$) differences in root surface area values of cucurbit rootstocks determined on the 30th day. In this period, root surface areas varied between 832.47 - 1142.65 cm^2 (Fig. 4). The average root surface area was found to be 1076.76 cm^2 in intraspecific rootstocks, 954.44 cm^2 in interspecies rootstocks and 897.67 cm^2 in commercial rootstocks. The highest root surface areas were seen in RS2 (1142.65 cm^2), RS1 (1134.93 cm^2), RS12 (1123.81 cm^2), RS3 (1103.00 cm^2) and RS6 (1102.03 cm^2) rootstocks and the lowest root surface areas were recorded in RS8 rootstock candidate (837.42 cm^2) and Gürdal commercial rootstock (865.00 cm^2). The total root surface area of Pandora F1 melon cultivar used as a scion was measured as 679.07 cm^2. It was determined that cucurbit rootstocks grafted with Pandora melon cultivar had significantly higher total root surface area values than the non-grafted Pandora cultivar. In general, intraspecific hybrid rootstocks had higher root surface areas than interspecies hybrid rootstocks. Root surface area increase was very slow in all rootstocks and scion in the first 10-day period from planting, it was higher in the second 10-day period as compared to the first period and it was quite rapid in the last 10-day period.

There were significant differences ($P \leq 0.01$) in root volume of rootstocks (Fig. 5). Interspecies hybrid rootstock RS12 and interspecies hybrid rootstock RS4 had the greatest performance in terms of root volume values.

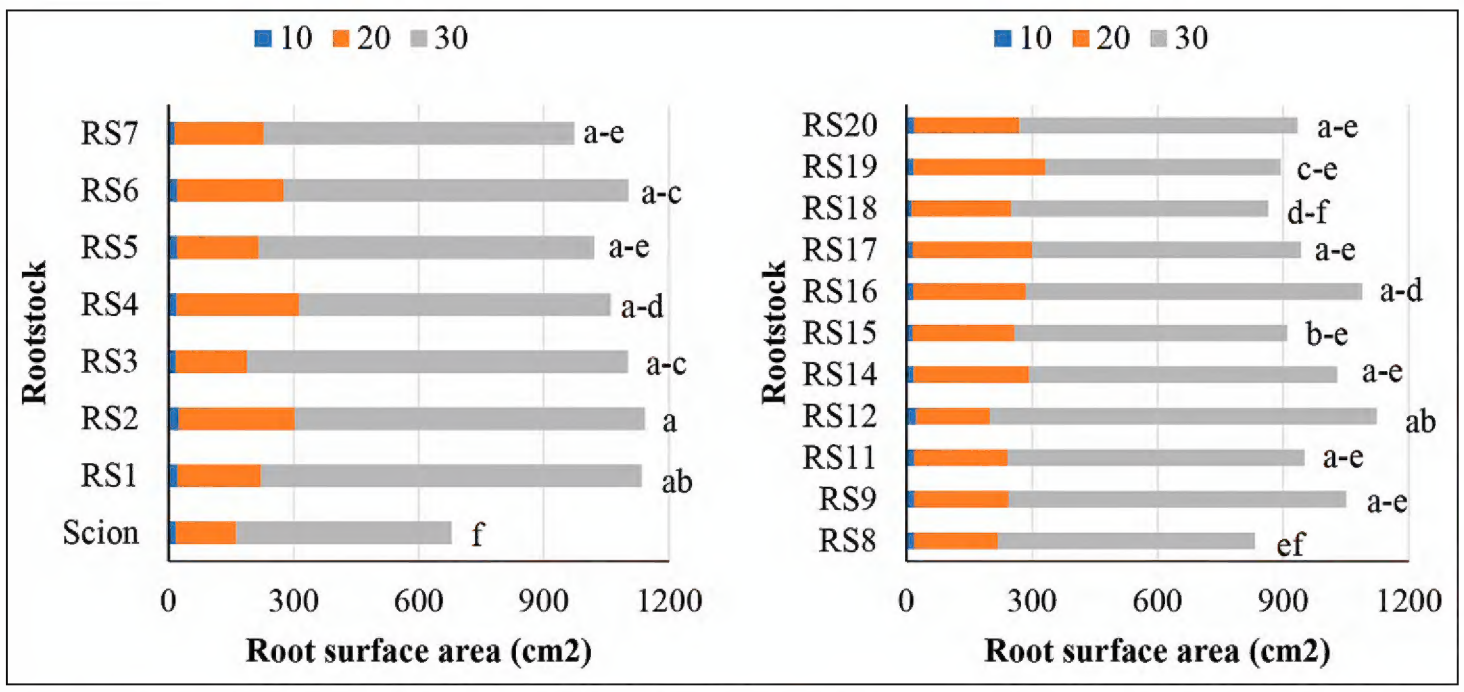

Fig 4. Root surface area (cm^2) of intraspecific (left) and interspecific (right) pumpkin rootstocks 10, 20 and 30 days after planting.

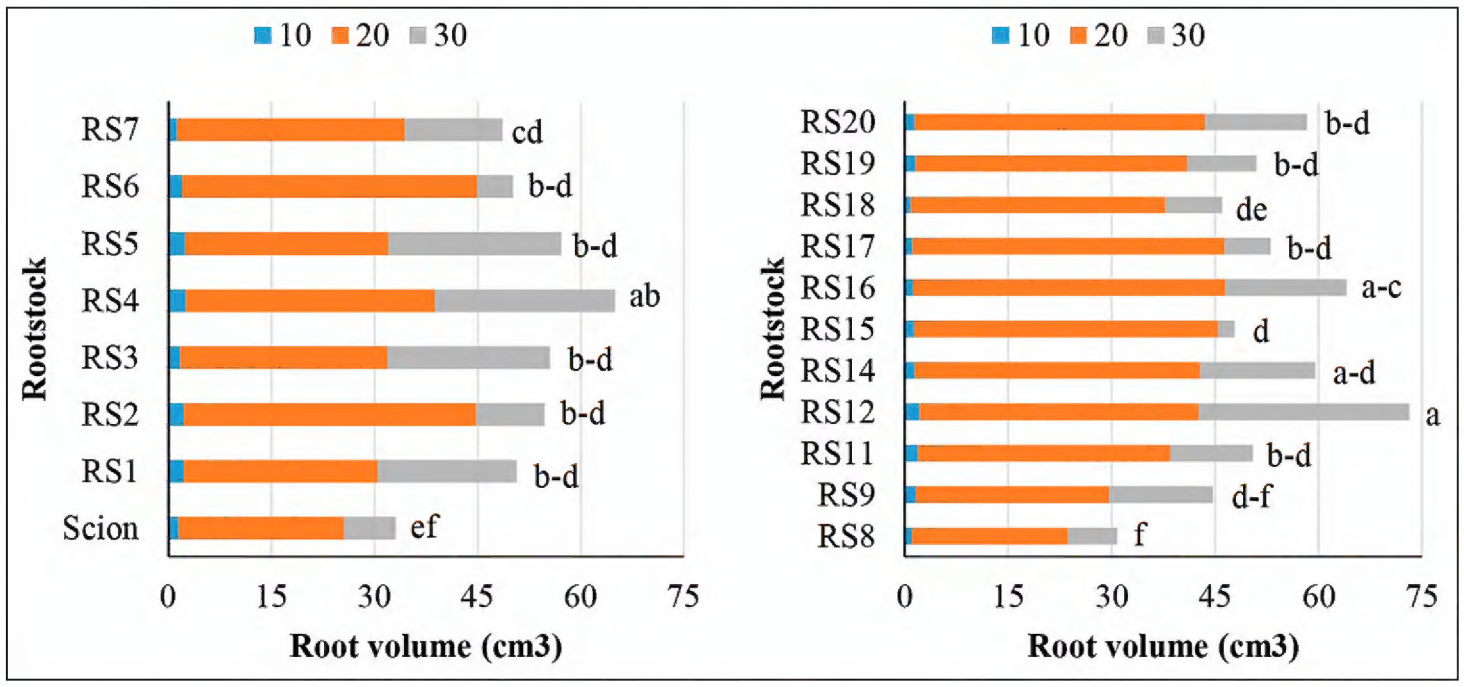

Fig 5. Root volume (cm^3) of intraspecific (left) and interspecific (right) pumpkin rootstocks 10, 20 and 30 days after planting.

RS8 (30.80 cm^3) rootstock, which recorded the lowest root volume value, had 137.5% lower root volume capacity than RS12 (73.15 cm^3) rootstock. When the change in root volume values of rootstocks was examined in three 10-day periods, it was seen that there was a very slow growth in the first 10 days from planting, the growth in the second 10 days increased considerably and the growth slowed down in the last 10 days (Fig. 5).

Yetişir and Karaca (2018) investigated rooting levels of 8 zucchinis, 2 commercial zucchini rootstocks (Macis and Argentario) and 4 *C. maxima* × *C. moschata* rootstock varieties (Kublai, Ferro, Kazako and RS841) collected from the Mediterranean Basin. Inclined cut grafting was applied and rooting status, root length, root thickness and root weights were determined in plants uprooted 30 days after grafting. Commercial zucchini rootstocks had higher rooting degree and root thickness values. The longest root length was determined in hybrid cultivars. Local zucchini genotypes were prominent for root dry weight. It was determined that rooting characteristics of local zucchini rootstocks were similar to commercial rootstocks.

A linear relationship was reported between root and shoot biomass because of the functional balance between root activity and photosynthesis (Atkinson, 2000). Strong root system of rootstocks provides more cytokine production and easier water and ion uptake. There are studies showing that rootstocks with strong root development made a positive contribution to growth and yield (Colla et al., 2010; Koevoets et al., 2016). In this study, leaf, stem and root dry weights of the rootstocks were also determined on the 30th day from planting and it was determined that there were important differences among the rootstocks.

Leaf dry weights varied between 2.08 - 3.24 g, stem dry weights between 1.21 - 1.62 g and root dry weight between 0.34 - 0.77 g. RS19 and RS16 rootstocks had greater performance for leaf dry weight, RS16 and RS12 for stem dry weight and RS12 and RS11 rootstocks for root dry weight than the other rootstocks. Average leaf weight of intraspecific hybrid rootstocks (2.52 g) was lower than that of interspecies hybrid rootstocks (2.63 g) and commercial rootstocks (2.60 g). Intraspecific hybrid rootstocks also

had greater average root weight values than the other two groups.

Edelstein et al. (2004) investigated the vegetative growth parameters of Arava F1 melon cultivar grafted on 22 different cucurbit rootstocks. Intraspecific (*C. moschata* × *C. moschata*) and interspecies (*C. moschata* × *C. argyrosperma*, *C. argyrosperma* × *C. moschata and C. maxima* × *C. moschata*) hybrid genotypes were used as rootstocks. In terms of vegetative growth, *C. moschata* × *C. moschata* rootstocks were identified as the most suitable rootstocks for melon.

CONCLUSION

Present findings revealed that intraspecific and interspecies local cucurbit rootstocks developed within the rootstock breeding program could reliably be used as commercial rootstocks to obtain grafted melon seedlings. Investigation of root architecture and phenotypic selection of rootstocks of several vegetable species are the primary interest in rootstock breeding programs. In this study, the relationships between root architectures of rootstock candidates and different rootstock/scion combinations were presented in detail. When the investigated traits were evaluated together, it could be stated that RS1, RS2, RS4 and RS11 rootstocks performed better in general. Research on the effects of all rootstock candidates (RS1-RS17) used in this study on yield components and fruit quality in grafted melon cultivation continues. In the near future, after all traits (rootstock/scion compatibility, graft success, vegetative growth, development, earliness, yield and quality, etc.) were evaluated together, new local rootstock candidates for grafted melon will be selected and registered as a variety.

Conflict of interest

The author declare no conflict of interest.

Author contribution

Kandemir, D. responsible for designing the study, collecting the data, conducting statistical analyses, and writing, reviewing, and correcting the manuscript.

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